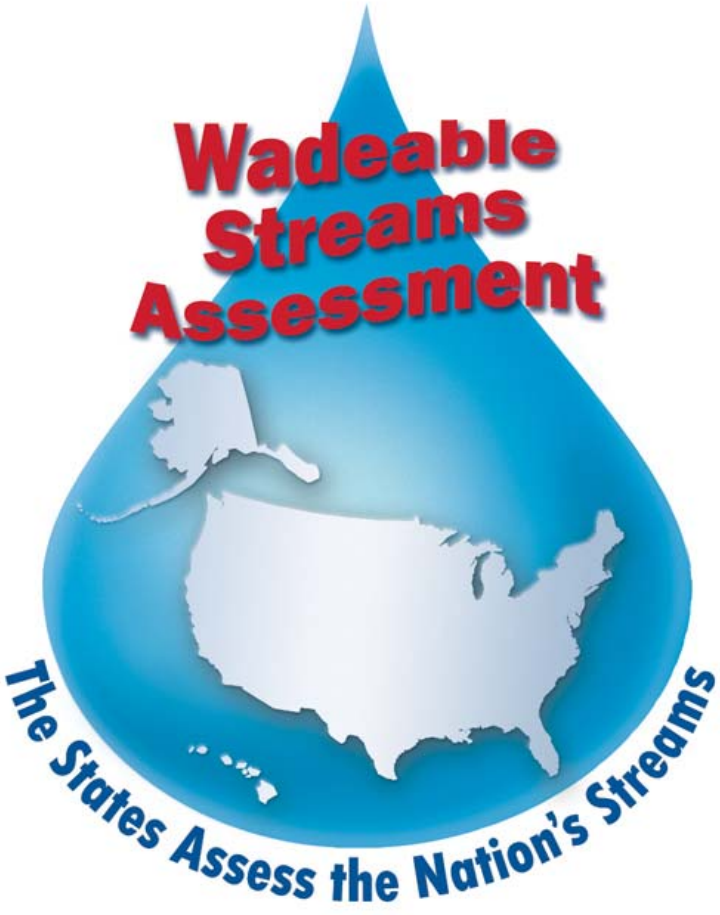
United States Environmental Protection Agency
Office of Water
Office of Environmental Information
Washington, DC

Wadeable Streams Assessment

Benthic Laboratory Methods



July 2004

FINAL

NOTICE

The intention of the WSA project is to provide a comprehensive "State of the Streams" assessment for streams across the United States. The complete documentation of overall WSA project management, design, methods, and standards is contained in five companion documents, including:

- *National Wadeable Streams Assessment: Integrated Quality Assurance Project Plan*
- *National Wadeable Streams Assessment: Site Evaluation Guidelines*
- *National Wadeable Streams Assessment: Field Operations Manual*
- *National Wadeable Streams Assessment: Benthic Laboratory Methods*
- *National Wadeable Streams Assessment: Water Chemistry Laboratory Manual*

This document (*Benthic Laboratory Methods*) contains information on the methods for analyses of the water samples to be collected during the project, quality assurance objectives, sample handling, and data reporting. These methods are based on the guidelines developed and followed in the Western Environmental Monitoring and Assessment Program (Peck et al. 2003). Methods described in this document are to be used specifically in work relating to WSA. All Project Cooperator laboratories should follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. More details on specific methods for site evaluation, sampling, and sample processing can be found in the appropriate companion document.

The suggested citation for this document is:

> USEPA. 2004. Wadeable Stream Assessment: Benthic Laboratory Methods. EPA841-B-04-007. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.

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1.0 SORTING AND SUBSAMPLING

Scope and Applicability: This procedure is to be used to facilitate processing and identification of benthic organisms collected in freshwater wadeable streams.

1.1 Responsibility and Personnel Qualifications: This procedure may be used by any person who has received training in processing and identification of benthic macroinvertebrates. A laboratory staff member qualified to perform quality control (QC) checks must be present when samples are processed by an inexperienced individual, or when QC checks are needed for 10% of an experienced sorter's samples. The qualifications of this individual include achieving $90% sorting efficiency. The roles and responsibilities of the QC Officer are described below.

- Provides oversight of daily operations and sample processing, monitors QC activities to determine conformance, and conducts performance and systems audits of the procedures.

- Verifies the completeness of every Benthic Macroinvertebrate Laboratory Bench Sheet (Attachment 1) to ensure header information is correctly entered.

- Checks sorted grids of all inexperienced laboratory personnel (those who have not achieved a $90% sorting efficiency) for missed organisms and records the number of missed organisms in the appropriate blank of the Benthic Macroinvertebrate Laboratory Bench Sheet.

- Checks 10% of an experienced individual's samples.

- Determines the sorting efficiency for each sample and sorter. The sorter's sorting efficiency
  is recorded on the bench sheet.

- Performs evaluations to ensure that QC is maintained throughout the laboratory sorting and subsampling procedure. Evaluations include double-checking work as it is completed and providing written documentation of these reviews to ensure that the standards set forth in the QAPP are met or exceeded.

1.2 Precautions:

1. Because it can be difficult to detect the organisms in stream samples (due to inexperience, detritus, etc.), a QC check must be performed by a person who has received instruction by senior biology staff familiar with processing benthic samples.

2. The QC checks in the Pertinent QA and QC Procedures section must be performed only by qualified personnel (QC Officers). These QC checks must be performed immediately following sorting of each grid.

3. Wear appropriate clothing for safety precautions, such as nitrile gloves, rubber apron, long pants, etc.

4. Be sure that all sorting equipment is thoroughly cleaned and free of organisms prior to sorting the next sample.

1.3 Equipment/Materials:

U.S. 35 sieve (500 :m or smaller)
Round buckets
Standardized gridded screen (370-:m mesh screen, 30 squares, each 6 cm^2)[1]
White plastic holding tray for gridded screen[1]
6 cm scoop
6-cm^2 metal dividing frame ("cookie cutter")
White plastic or enamel pan (6" x 9") for sorting
Scissors
Teaspoon
India ink pens
Dropper
Forceps
Specimen vials, caps or stoppers
Sample labels
Illuminated magnifier
Dissecting microscope for organism identification with fiberoptics light source (10–40×)
70-80% denatured ethanol
Benthic Sample Log-In form
Benthic Macroinvertebrate Laboratory Bench Sheet
Stereo zoom microscope (10x)

1.4 Procedure:

1.4.1 General

1. Receipt of samples must be recorded in the laboratory on the Benthic Sample Log-In form (Attachment 2). Assign the appropriate chronological bench number to each sample. Store samples at room temperature until ready for processing.

2. Sample container(s) will arrive with very little alcohol to expedite shipping times and to account for hazardous material handling requirements. Refill the sample container(s) with 70-80% ethanol on THE SAME DAY THEY ARE RECEIVED in the laboratory. After the additional alcohol is in the sample, store it until sorting begins.

3. Sort and preserve a randomized 500-organism subsample separately from the remaining sample using a gridded screen.

4. Document the level of effort, or proportion of sample processed, on the Benthic Macroinvertebrate Laboratory Bench Sheet (Attachment 1) for each sample as it is subsampled and sorted.

5. Record the following information on internal sample labels used for vials of sorted material with India ink pen on cotton rag paper or an acceptable substitute.

Station Name
Station Location
Station Number
Date Sorted
Sorter's Initials
"1 of 2" or "2 of 2" if necessary

[1]Some Cooperators may choose not to use the gridded screen in a plastic holding tray

1.4.2 Subsampling

1. Remove the lid from the sample container(s) and pull out the internal sample label (save the sample label—it will need to be returned to the sample container with the archived portion of the sample that does not get processed). Record sample collection information on a Benthic Macroinvertebrate Laboratory Bench Sheet. Header information required includes station name, station location, station number, project name, bench number, sample type, date the sample was collected, and the field team who collected the sample (e.g., Team 1). Set the bench sheet aside.

2. Carefully decant the alcohol from the sample container by pouring the fluid through a sieve (U.S. 35) into a separate container (the alcohol is saved to preserve the archived portion of the sample that does not get processed). Inspect the mesh of the sieve for any organisms and return organisms found to the sample.

3. Transfer the homogenized sample material to the gridded screen portion of the grid (use more than one subsampling device if necessary). Wash the sample thoroughly by running tap water over it to remove any fine material. If there is more than one jar for any particular sample, empty and wash each jar onto the Caton-type grid one at a time, making sure to spread each jar's contents evenly across the tray.

4. Place the gridded screen into the larger white tray. (Note: Some Cooperators may not use the gridded screen and holding tray). Add enough water to spread the sample evenly throughout the grid (the water level should be relatively close to the top of the white tray). Spread the sample material over the bottom of the pan as evenly as possible. Move the sample into the corners of the pan using forceps, spoon, or by hand. Vibrate or shake the pan gently to help spread the sample.

5. Lift the screen out of the white tray to drain. Pour off or siphon excess water from the white tray and set the screen back into the tray. Leave just enough water in the bottom of the tray so that it barely covers the screen once it is returned to the tray to allow the sample to remain moist.

6. Use a random number generator to select at least 4 grids to process (select one letter and one number, e.g., A-5, F-2). Four grids are sorted from the sample to ensure that the subsample material is representative of the overall stream sample. Remove all the material using the following procedure from that grid and place the removed material into a separate holding container, such as a white plastic or enamel pan. If two trays are being used to hold a large sample, the same grid on the second pan will also be removed. Continue until the material from all 4 grids is removed. The material is removed as follows:

    a. Place the metal dividing frame or "cookie cutter" over the sample at the approximate location of the grid selected for processing (based on the letters and numbers marked on the sides of the gridded tray). Use a pair of rulers or other straight edges to facilitate lining up the cookie cutter at the intersection if necessary.

    b. Remove the material within the "cookie cutter" using the 6-cm scoop, a teaspoon, forceps, or dropper. Depending on the consistency of what is in the sample, it might be necessary to cut the material along the outside of the "cookie cutter" with scissors or

separate it with forceps so that only one grid's worth of sample material is used. Inspect the screen for any remaining organisms. Use the following rules when dealing with organisms that lie on the line between two grids:

- An organism belongs to the grid containing its head.
- If it in not possible to determine the location of the head (i.e., for worms), the organism is considered to be in the grid containing most of its body.

- If the head of an organism lies on the line between two grids, all organisms on the top of a grid and those on the right side of a grid belong in that grid, and are picked with that grid.

c. Place the material from the selected grid(s) into a separate white plastic or enamel pan. Add the necessary amount of water to the pan to facilitate sorting.

d. Set the subsampling device aside in case more grids need to be retrieved later. Cover the sample with aluminum foil to prevent desiccation of the sample and damage to specimens (periodically moisten the sample with water from a spray bottle if the top layer begins to dry). Between each subsampling operation, be careful not to disturb the subsampling device to prevent redistribution of specimens, which could possibly change the probability of selection.

NOTE: More than one grid *can* be removed before sorting. If desired, place the sample removed from each selected grid into a separate pan with water while awaiting processing to prevent drying out and damage to specimens. Place a label in each pan to properly identify each grid. It is also acceptable to combine several grids for sorting if it is determined or thought that the organisms/grid is sufficiently low enough and will not result in a subsample in excess of the upper limit of the target number.

1.4.3 Sorting

1. Remove the macroinvertebrates from the material of each grid until the target number of organisms is attained (e.g., 500). If it is apparent that the target number will be substantially exceeded by sorting the entire final grid, that grid may be respread in another gridded screen and sorted until 500-550 total organisms are removed from the sample.

   a. Remove the macroinvertebrates from the detritus by using forceps or by transferring a spoonful of the material to a petri dish for examination beneath a dissecting microscope; place picked organisms in an internally labeled, vial (or larger container, if necessary) containing 70-80% denatured ethanol as a preservative.

   b. Keep a count of the number of organisms removed and enter the number of organisms found in each grid under that column on the Benthic Macroinvertebrate Laboratory Bench Sheet. Enter the sorter's initials in the appropriate column on the bench sheet for each grid that is sorted.

   c. If the grid does not yield the target number of organisms (including organisms collected during QC checks), randomly select another grid and continue with the above protocol. Ensure that at least 500 organisms are attained.

d. If 150 or more organisms are found in the first grid during the first half-hour of sorting, collect the three other chosen grids and respread into a different gridded screen and sort that portion of the sample (i.e., the 4 grids) following steps a through c.

e. Do *not* remove or count empty snail or bivalve shells, specimens of surface-dwelling or strict water column[2] arthropod taxa (e.g., Collembola, Veliidae, Gerridae, Notonectidae, Corixidae, Cladocera, or Copepoda), or incidentally-collected terrestrial taxa. Also, *do not* count fragments such as legs, antennae, gills, or wings. For Oligochaeta, attempt to remove and count *only* whole organisms and fragments that include the head; also, *do not* count fragments that do not include the head.

2. Each grid, once it is picked by the initial sorter, must be checked for missed organisms before another grid is processed. This step is performed by an experienced, certified, laboratory QC Officer, as detailed below. Any missed organisms found by the QC Officer will be counted and placed into the sample vial, or other suitable sample vial, and the number of organisms missed will be noted on the Benthic Macroinvertebrate Laboratory Bench Sheet and added to the final count of the sample.

3. Once the QC check of the material in the pan has been completed, it is removed from the pan and placed in a separate container with preservative (70-80% ethanol). The container should be labeled “Sorted Residue”, on both internal and external labels (“Sorted Residue” will include material from *all* grids processed for each sample). Internal sample labels should be made of cotton rag paper or an acceptable substitute, recording the same information as before.

4. After the QC check is completed, and the target number has been reached, search the entire tray for 5-10 minutes, looking for large/rare organisms (Vinson and Hawkins, 1996). Large/rare is defined as any organism larger than ½" long and found in less than 1/8 of the tray holding the entire sample. Place any organisms found into a vial labeled “L/R” for “Large/Rare”.

5. All material not subsampled (remaining on the grid) must be returned to the original container with the preservative. This container should include the original sample labels and a separate label “Unsorted Sample Remains” should be added inside the container and on the outside. The lid should be tightly closed and the container placed in archiving until **all** appropriate QC checks are completed (subsampling and taxonomy), after which it is discarded.

6. Record the sorting date each sample was completed near the top right corner of the bench sheet.

1.5 Pertinent QA and QC Procedures:

1. Initially, experienced QC Officers will use microscopes to check **all** sorted grids from the first five samples processed by a sorter to ensure that all organisms were removed from the

[2]Strict water column taxa are those that do not have at least one life stage that is benthic (i.e., bottom-dwelling).

detritus. This will not only apply to inexperienced sorters, but also to those initially deemed as "experienced." Qualification will only occur when sorters are consistent in achieving $90% sorting efficiency after at least five samples have been checked. Experienced QC officers will check samples using up to 10x magnification.

2. The QC Officer will calculate percent sorting efficiency (PSE) for each sample as follows:

$$PSE = \frac{A}{A + B} \times 100$$

where A = number of organisms found by the primary sorter, and B = number of recoveries (organisms missed by the primary sort and found by the QC check).

If the sorting efficiency for each of these five consecutive samples is $90% for a particular individual, this individual is considered "experienced" and can serve as a QC Officer. In the event that an individual fails to achieve $90% sorting efficiency, they will be required to sort an additional five samples in order to continue to monitor their sorting efficiency. However, if they show marked improvement in their sorting efficiency prior to completion of the next five samples, whereby they acquire the $90% sorting efficiency, the QA Officer may, at his/her discretion, consider this individual to be "experienced." Sorting efficiency should not be calculated for samples processed by more than one individual.

3. After individuals acquire a $90% sorting efficiency, 10% (1 out of 10) of their samples will be checked.

4. If an "experienced" individual fails to maintain a $90% sorting efficiency as determined by QC checks, QC checks will be performed on every grid of five consecutive samples until a $90% sorting efficiency is achieved. During this time, that individual will not be able to perform QC checks.

2.0 TAXONOMY

Scope and Applicability: This procedure is to be used to facilitate identification of benthic macroinvertebrate organisms collected in freshwater wadeable streams.

2.1 Responsibility and Personnel Qualifications: This procedure may be used by any person who has received training in identification of freshwater benthic macroinvertebrates, i.e., taxonomy. It is also important that the taxonomist maintains contact with other taxonomists through professional societies and other interactions, and keeps up with the pertinent literature, since systematics and species identifications change over time. A second taxonomist will be checking 10% of the identifications of the primary taxonomist for quality control (QC), as noted below, to ensure agreement in the identifications and minimize problems during the project. Samples will be sent to the taxonomists on a regular basis during the project as subsampling of the field samples is completed to avoid delays in identifying the organisms.

2.2 Precautions:

1. Identifications need to be based on current published taxonomic references.

2. If technical literature citations specifying nomenclatural validity are not available or otherwise are unknown, taxon names from the Integrated Taxonomic Information System (ITIS), available on the Web at http://www.itis.usda.gov/, are to be used.

3. A list of primary and secondary technical literature used in completing the identifications must be prepared and submitted to the Tetra Tech project facilitator when samples are returned (see below).

2.3 Equipment/Materials:

Stereo dissecting microscope (e.g., Nikon SMZ-U, 7.5-75 x, or similar) with fiberoptics light source
Compound microscope (e.g., Nikon Labophot 2, 60-1500 x, or similar)
Petri dishes
Microscope slides (1" x 3" flat, precleaned)
Cover slips (appropriately sized)
CMCP-10 (or other appropriate mounting medium)
India ink pens
Dropper
Forceps
Specimen vials, with caps or stoppers
Sample labels
70-80% denatured ethanol in plastic wash bottle
Benthic Macroinvertebrate Taxonomic Bench Sheet
Hand tally counter

2.4 Procedure:

1. On receipt of a set of sample vials from the project cooperator or contractor laboratory, remove the chain-of-custody form from the shipping container, sign and date it to verify that the samples were received (in the “received by” space. Compare all sample numbers on the form with those entered on the labels of samples that actually were in the shipment. If any vials were broken, notify the project facilitator immediately. Maintain the chain-of-custody form with the samples; it will be needed to return the samples.

2. Empty one sample vial at a time into a small petri dish. Add 80% denatured ethanol to keep the organisms covered. Remove the internal sample label and complete the top portion of a Benthic Macroinvertebrate Taxonomic Bench Sheet (Attachment 3), using the information from the label or that provided by the project facilitator.

3. Begin by viewing the sample under the stereo dissecting microscope and removing similar organisms to other dishes (keep covered with 80% ethanol). As organisms are identified to the correct taxon level for the project (usually genus, Attachment 4), record the identifications on the Benthic Macroinvertebrate Taxonomic Bench Sheet (under taxon). Enter the number of larvae, pupae, and adults of each taxon under those columns on the bench sheet. Also enter the Taxonomic Serial Number (found in ITIS). A taxonomic list can be downloaded from ITIS to be compared to what each lab finds (http://www.itis.usda.gov/taxmatch_ftp.html).

4. Prepare slide mounts of Chironomidae and Oligochaeta as needed using CMCP-10 (or CMC-9, CMC-10, or other media) and applying a coverslip. View these organisms under the compound microscope to ensure that all necessary diagnostic characters have been observed, according to the taxonomic key or other literature. Record the identifications on the bench sheet as above. The slides should be labeled with the sample number or log-in number.

5. Prepare a list of primary and secondary technical literature used in completing the identifications. Provide complete citations in bibliographic format, including authors' names, date of publication, title of document, name of journal or publisher, volume and page numbers, or ISBN number, as appropriate.

6. If ***damaged organisms*** can be identified, they are counted ONLY if:

   (a) the fragment includes the head, and, in the case of

   • arthropods, the thorax
   • oligochaetes, a sufficient number of segments

   (b) the mollusk shell (bivalve or gastropod) is occupied by a specimen

   (c) the specimen is the sole representative of a taxon in the sample

7. If ***early instar or juvenile*** specimens can be identified, they are counted ONLY if:

   (a) with confidence, they can be associated with one or more mature specimens that have a more developed morphology

   (b) the specimen is the sole representative of a taxon in the sample

8. Enter a taxonomic certainty rating (from 1 to 5, most certain to least certain) for each taxon identified on the bench sheet (under the column "TCR"). Also enter the number of the reference collection specimen(s) used in the identification or prepared for this project under that column on the bench sheet.

9. Add the number of organisms from each developmental stage and enter the total on the bench sheet.

10. Complete the bench sheet by entering totals for each developmental stage and the total number of each taxon in the cells at the bottom of the sheet. Cross-check to be sure the totals were summed correctly. Make a copy of the bench sheet for the project file.

11. Create a reference collection with at least one specimen from each genus (or lowest taxonomic level IDed). When a sample is chosen to be the source of specimen(s) to represent a name in the master taxa list, the appropriate specimen(s) in that sample representing the concept of that taxon to the taxonomist should be removed and placed in the reference collection. Labels will be placed in the primary sample container indicating the placement of any specimen(s) removed for the reference collection. Circle slide-mounted specimens with a grease pencil (or other appropriate mark) to indicate those

which belong to the reference collection. For all slides containing reference and non-reference specimens, be sure to place a label in the sample container that **does not** contain the reference collection. Each laboratory should maintain a master list of taxa recorded. The project facilitator will coordinate any necessary inter-lab communication and produce and integrated master taxa list for the project.

12. Carefully return the rest of the organisms to the original sample vial, fill with 70-80% denatured ethanol and cap tightly.

13. Re-package the samples and slide mounted specimens carefully, and sign and date the chain-of-custody form in the next "relinquished by" space. The samples should be shipped, properly packed in a box, by overnight carrier to the project facilitator, and receipt confirmed by the person doing the shipping. Each taxonomist should retain a full set of bench sheet copies, and ship the original bench sheets in an envelope to the project facilitator. Samples and bench sheets should be shipped separately.

2.5 Pertinent QA and QC Procedures:

1. On receipt of the samples, the project facilitator will randomly select 10% of the samples to be sent to the QC taxonomist, another experienced taxonomist who did not participate in the original identifications. A chain-of-custody form will be completed and sent with the samples.

2. The QC taxonomist will perform whole-sample re-identifications, with care taken to ensure inclusion of all slide-mounted specimens, completing another copy of the Benthic Macroinvertebrate Taxonomic Bench Sheet for each sample. Each bench sheet should be labeled with the term "QC Re-ID." As each bench sheet is completed, it should be faxed to the project facilitator.

3. The project facilitator will compare the taxonomic results (counts AND identifications) generated by the primary and QC taxonomists for each sample and calculate percent disagreement in enumeration (PDE) and percent taxonomic disagreement (PTD) as measures of taxonomic precision as follows:

$$PDE = \frac{|n1 - n2|}{n1 + n2} \times 100$$

where $n1$ is the number of specimens counted in a sample by the first taxonomist and $n2$ is the number of specimens counted by the QC taxonomist.

$$PTD = \left[1 - \left(\frac{comp_{pos}}{N} \right) \right] \times 100$$

where $comp_{pos}$ is the number of agreements (positive comparisons) and N is the total number of pecimens in the larger of the two counts.

4. Unless otherwise specified by project goals and objectives, the measurement quality

objective for enumerations will be a mean PDE less than or equal to 5 and a mean PTD less than or equal to 15, calculated from all the samples in the 10% set sent to the QC taxonomist. Results greater
than these values will be considered acceptable for the dataset overall, although individual sample results in excess should be examined for indications of error patterns.

5. Corrective action will include determining problem areas and consistent disagreements, addressing problems through taxonomist interactions. Disagreements resulting from identification to a specific taxonomic level, creating the possibility to double-count "unique" or "distinct" taxa will also be rectified through corrective actions.

6. A report or technical memorandum will be prepared by the project facilitator. This document will quantify both aspects of taxonomic precision, assess data acceptability, highlight taxonomic problem areas, and provide recommendations for improving precision. This report will be submitted to the client, with copies sent to the primary and QC taxonomists and another copy maintained in the project file.

3.0 REFERENCES

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Peck, D.V., J.M. Lazorchak, and D.J. Klemm (editors). Unpublished draft. *Environmental Monitoring and Assessment Program - Surface Waters: Western Pilot Study Field Operations Manual for Wadeable Streams*. EPA/XXX/X-XX/XXXX. U.S. Environmental Protection Agency, Washington, D.C.

Stribling, J.B., S.R. Moulton, and G.T. Lester. 2003. Determining the Quality of taxonomic data. *Journal of the North American Benthological Society* 22(4):621-631.

Vinson, M.R. and C.P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. *Journal of the North American Benthological Society* 15(3): 392-399.

ATTACHMENT 1

Benthic Macroinvertebrate Laboratory Bench Sheet

BENTHIC MACROINVERTEBRATE LABORATORY BENCH SHEET (FRONT)

Project Name/Number _____

Serial ID# _____ Waterbody Name _____

Sorter _____ Station ID _____

Sort Date _____ Collection Date _____

GRID ORDER	RANDOM NUMBER GRID ID	NUMBER OF INDIVIDUALS PER GRID	CUMULATIVE NUMBER OF ORGANISMS
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			

Check off grids as selected:

	1	2	3	4	5	6
1						
2						
3						
4						
5						

BENTHIC MACROINVERTEBRATE LABORATORY BENCH SHEET (BACK)

SUBSAMPLING/SORTING INFORMATION

Sorter _____

Date _____

Number of grids picked: _____

Time expenditure _____ No. of organisms _____

Indicate the presence of large or obviously abundant organisms:

QC: ❑ YES ❑ NO **QC Checker** _____

# organisms originally sorted		(# organisms recovered by checker	+	# organisms originally sorted)	=	% sorting efficiency
	÷		+		=	

≥90%, sample passes _____

<90%, sample fails, action taken _____

_____

TAXONOMY

ID _____

Date _____

Explain TCR ratings of 3-5:

Other Comments (e.g. condition of specimens):

QC: ❑ YES ❑ NO **QC Checker** _____

Organism recognition ❑ pass ❑ fail

Verification complete ❑ YES ❑ NO

General Comments (use this space to add additional comments):

_____

_____

_____

_____

_____

_____

_____

_____

ATTACHMENT 2

Benthic Sample Information Report

(This page is intentionally blank.)

SITE_ID	DATE_COL	VISIT_NO	SAMPTYPE	SAMP_ID	LAB_ID
Site ID	Sample Collection	Within Year	Benth. Samp. Type	Sample Tracking Number	Lab Sample
	Date	Site Visit Number		from Jar Label	ID Number

SITE_ID	JARS_COL	JARSRECD	DATE_REC	PCTCOUNT	COM_LAB
Site ID	Number of	Number of	Date Samples	Percent of Sample	Lab Personnel
	Jars Collected	Jars Received	Received	Counted	Comments

ATTACHMENT 3

Benthic Macroinvertebrate Taxonomic Bench Sheet - Excel Spreadsheet Example

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Electronic Benthic Taxonomic Bench Sheet

SITE_ID	DATE_COL	VISIT_NO	SAMPTYPE	SAMP_ID	LAB_ID	LAB_TAXON
Site ID	Sample Collection	Within Year	Benthic Sample Type	Sample Tracking Number	Lab Sample	Lab Taxa ID Number
	Date	Site Visit Number		from Jar Label	ID Number	

SITE_ID	TAXANAME	TOTAL	LARVAE	PUPAE	ADULTS	TCR	PHYLUM	CLASS
Site ID	Unique Taxon Name	Number of	Number of Larvae	Number of Pupae	Number of Adults	Taxonomic	Taxonomic	Taxonomic
		Individuals				Certainty Rating	Phylum	Class
		3	1	1	1			
		0						
		0						
		0		

Electronic Benthic Taxonomic Bench Sheet

SITE_ID	ORDER	FAMILY	SUBFAM	TRIBE	GENUS	SPECIES	SERIAL	DISTINCT	LR_TAXA
Site ID	Taxonomic	Taxonomic	Taxonomic	Taxonomic	Taxonomic	Taxonomic	ITIS Serial	Distinct Taxa	Large/Rare
	Order	Family	Subfamily	Tribe	Genus	Species	Number	Within Sample (Y/N)	Taxa (Y/N)

SITE_ID	COM_LAB
Site ID	Lab Personnel
	Comments

ATTACHMENT 4

Taxonomic Level of Effort

This is the Standard Taxonomic Effort list for benthic macroinvertebrates. It represents the minimum level needed for mature and well preserved specimens. The lowest targeted taxonomic level will be genus. Due to taxonomic limitations, some groups cannot be identified to the genus level and therefore should be taken to the level specified below. Foe all taxonomic groups, if the level can easily go lower, for example monotypic genera, or if only one genus or species is known to occur in a certain geographic area, then these specimens should be identified at the lowest possible taxonomic level (e.g., Ephemerellidae *Drunella doddsI*). If the minimum taxonomic level cannot be achieved due to immature, damaged, or pupal specimens this houlsd be noted in the data file "flag" variable (e.g., IM = y, DD = y, PP = y). If a unique taxon is determined for which the appropriate taxonomic level is not available in the literature and there are other taxa in that taxonomic level, these specimens shall be given a code of UN = y (e.g., Ephemerellidae *Drunella doddsi* and *Drunella* sp. UN = y vs. *Drunella* sp. UN = n) so that these specimens can be distinguished from specimens that are NOT unique and are to be grouped at a higher taxonomic level due to imprecise identification.

PHYLUM ANNELIDA

Class Branchiobdellida	Identify to genus
Class Hirudinea	Identify to genus
Class Oligochaeta	Identify to family
Class Polychaeta	Identify to family

PHYLUM ARTHROPODA

Class Arachnoidea	
Acari	Identify to family
Class Insecta	
Coleoptera	Identify to genus
Diptera	Identify all to genus except in the following cases:
Chironomidae	Identify to genus (this may not be possible for some groups which should be identified to at least tribe or subfamily)
Dolichopodidae	Identify to family
Phoridae	Identify to family
Scathophagidae	Identify to family
Syrphidae	Identify to family
Ephemeroptera	Identify to genus
Hemiptera	Identify to genus
Lepidoptera	Identify to genus
Megaloptera	Identify to genus
Odonata	Identify to genus
Plecoptera	Identify to genus
Trichoptera	Identify to genus
Class Malacostraca	Identify to genus
Amphipoda	Identify to genus
Decapoda	Identify to genus
Isopoda	Identify to genus
Mysidacea	Identify to genus
Class Ostracoda	Identify to genus

PHYLUM COELENTERATA

PHYLUM MOLLUSCA

Class Bivalvia	Identify to genus
Class Gastropoda	Identify to genus except in the following cases: Hydrobiidae - Identify to family

PHYLUM NEMERTEA Identify to genus